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Peptide YY and glucagon-like peptide-1 contribute to decreased food intake after Roux-en-Y gastric bypass surgery

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Abstract

Background/Objectives: Exaggerated postprandial secretion of glucagon-like peptide-1 **(**GLP-1) and peptide YY (PYY) may explain appetite reduction and weight loss after Roux-en-Y gastric bypass (RYGB), but causality has not been established. We hypothesized that food intake decreases after surgery through combined actions from GLP-1 and PYY. GLP-1 actions can be blocked using the GLP-1 receptor antagonist Exendin 9-39 (Ex-9), while PYY actions can be inhibited by administration of a dipeptidyl peptidase-4 (DPP-4) inhibitor preventing the formation of PYY_{3-36}.

Subjects/Methods: Appetite regulating gut-hormones and appetite ratings during a standard mixed-meal test and effects on subsequent ad libitum food intake were evaluated in two studies: In s*tudy 1*, nine patients with type 2 diabetes were examined prospectively before and 3 month after RYGB with and without Ex-9. In s*tudy 2*, 12 RYGB-operated patients were examined in a randomized, placebo-controlled, cross-over design on four experimental days with: 1) placebo, 2) Ex-9, 3) the DPP-4 inhibitor, sitagliptin, to reduce formation of PYY_{3-36} and 4) Ex-9/sitagliptin combined.

Results: In *study 1*, food intake decreased by 35% following RYGB compared with before surgery. Before surgery, GLP-1 receptor blockage increased food intake but no effect was seen postoperatively, while PYY secretion was markedly increased. In *study*

2, combined GLP-1 receptor blockage and DPP-4 inhibitor mediated lowering of PYY_{3-36} increased food intake by ~20% in RYGB patients, while neither GLP-1 receptor blockage nor DPP-4 inhibition alone affected food intake, perhaps due to concomitant marked increases in the unblocked hormone.

Conclusions: Blockade of actions from **only** one of the two L-cell hormones, GLP-1 and PYY_{3-36}, resulted in concomitant increased secretion of the other, probably explaining the absent effect on food intake on these experimental days. Combined blockade of GLP-1 and PYY-actions increased food intake after RYGB, supporting that these hormones play a role in decreased food intake postoperatively.

Keywords

Appetite, Bariatric surgery, Ad libitum food intake, Exendin 9-39, Dipeptidyl peptidase-4 inhibitor, Weight loss, Oxyntomodulin.

Introduction

Roux-en-Y gastric bypass (RYGB) surgery induces large and sustainable weight loss in obese patients[1]. The precise mechanisms behind the weight loss remain to be fully elucidated; however, neither malabsorption of macronutrients[2] nor changes in whole body energy expenditure seem to play a major role[3,4]. Further, scintigraphic studies have revealed that food rushes through the alimentary limb into the small intestine at abnormally high rates, which seems incompatible with a mechanical restriction of food intake as a causal mechanism of weight loss[5,6]. Rather, exaggerated postprandial secretion of anorexigenic gut hormones, resulting from the altered gastrointestinal

anatomy, may be involved. Indeed, the post-prandial plasma concentrations of the appetite inhibiting hormones Peptide YY (PYY) and Glucagon-like peptide-1 (GLP-1) increase 10-20 fold after RYGB[7-9], but increased secretion of neurotensin, cholecystokinin (CCK) and oxyntomodulin (OXM)[10] is also observed, as well as decreased secretion of ghrelin − changes which may all promote reduced food intake. The contribution of the individual hormones for decreased food intake after RYGB has never been revealed, but inhibition of gut hormone secretion in general by administration of the somatostatin analogue, octreotide, leads to increased food intake after RYGB[11,12]. Since somatostatin affects virtually all functions in the gastrointestinal tract[11], this effect may not solely reflect the inhibition of gut hormone secretion and furthermore does not reveal the importance of the individual hormones. The most dramatic secretory changes after RYGB are seen for the secretion of GLP-1 and PYY, both of which have prominent appetite-inhibitory properties [14–16] and exhibit synergistic effects on appetite and food intake in non-operated individuals[15,17,18].

Here, we report results from two studies addressing more specifically the role of GLP-1 and PYY in regulation of appetite and food intake after RYGB. In *study 1*, we investigated the role of endogenous GLP-1 on appetite and ad libitum food intake using the GLP-1 receptor (GLP-1R) antagonist, Exendin 9-39 (Ex-9), before and after surgery in patients with type 2 diabetes. In *study 2*, we expanded the method to include, not only Ex-9 infusion as in *study 1*, but also specific DPP-4 inhibition (with sitagliptin) whereby the formation of active PYY_{3-36} from full-length PYY_{1-36} is inhibited; this limits PYY-mediated inhibition of food intake, since the 3-36 truncated form of PYY is highly selective for the Y2-receptor[19] transmitting appetite inhibition[14]. Thus, Ex-9 infusion, DPP-4 inhibition, a combination of the two, or placebo on different study days allows

us to dissect the individual and combined importance of GLP-1 and PYY_{3-36} for regulation of appetite and ad libitum food intake. Our hypothesis was that GLP-1 and PYY_{3-36} act in combination to decrease appetite after RYGB and that simultaneous inhibition of both hormones might be required to reveal a significant effect on ad libitum food intake (primary outcome).

Materials and Methods

Patients were recruited from the Bariatric Surgery Program at Hvidovre Hospital (Hvidovre, Denmark). Both studies were approved by the Municipal Ethical Committee of Copenhagen (*Study 1*: Reg. nr. H-A-2008-080-31742, *Study 2*: H-1-2013-131) and performed in accordance with the Helsinki declaration. Trials were registered with ClinicalTrials.gov (*Study 1*: NCT01579987, *Study 2*: NCT02336659). Written informed consent was obtained from all patients before entering the study.

Detailed results from both studies regarding glucose metabolism have been reported elsewhere[20,21], but here we present new data on ad libitum food intake, appetite ratings (using visual analog scaling) and secretion of appetite regulating hormones (PYY, CCK, OXM, glicentin).

Study design

***Study 1*:** Patients with type 2 diabetes, who met the criteria for bariatric surgery (age >25 years, BMI>35 kg/m^2, and mandatory preoperative weight loss of 8% of total body weight accomplished) were recruited, as previously described[20]. Exclusion criteria included anorectic or antithyroid medication within 3 months prior to experiments, fasting C-peptide < 700 pmol/l, history of allergic reactions to exenatide or signs of neuropathy. Patients were examined at two visits, before (pre) and 3 months (3 mo)

after RYGB. The study also included a visit 1 week after surgery to characterize glucose metabolism, but ad libitum meal testing was not possible at this time. Incretin based therapies were paused and treatment with insulin analogues was changed to NPH insulin 14 days prior to the first preoperative study day. All other antidiabetic agents were discontinued three days prior to first experimental day. Each visit included two experimental days comprising a fixed, standard liquid mixed meal followed by an ad libitum meal 4 hours later during randomized, patient-blinded primed-continous infusions of Ex-9 (Bachem AG, Bubendorf, Switzerland) (bolus: 43.3 nmol/kg; infusion: 900 pmol/kg/min) or isotonic saline (equivalent bolus and infusion volumes).

Patients met in the morning after an overnight fast, catheters were inserted into antecubital veins of both arms, fasting blood samples were drawn (-40 to -30 min) followed by initiation of Ex-9 or saline infusions (-30 min). Basal samples were drawn (-10 to 0 min), and at time 0 min, patients ingested a liquid meal (200 mL, Fresubin Energy Drink, 1260 kJ (50E% carbohydrate, 15E% protein, and 35E% fat), Fresenius Kabi GmbH, Bad Homburg, Germany) over 30 minutes. Blood was sampled frequently for 4 hours after meal start. Immediately after the last blood sample (240 min), an ad libitum meal consisting of thoroughly mixed pasta Bolognese (energy content 533 kJ/100 g (53E% carbohydrate, 14E% protein and 33E% fat) was served. Patients were placed in a quiet corner and were instructed to eat until feeling full. One glass of water (100 mL) was allowed with the meal. The meal was weighed before and after serving and the difference defined the ad libitum food intake.

***Study 2*:** Subjects who had undergone uncomplicated RYGB 3–12 months earlier with fasting plasma glucose < 7.0 mmol/l and HbA1c < 48 mmol/mol were included as previously described[21]. Exclusion criteria were similar as in study 1 plus allergy for

sitagliptin. Patients were examined in a randomized, placebo-controlled, single-blinded, cross-over design. On four different study days separated by at least 48 hours, patients underwent a standard mixed meal test followed 4 hours later by an ad libitum meal with a concurrent primed-continuous infusion of Ex-9 or saline combined with administration of either DPP-4 inhibitor or placebo tablets: The four study days were: (1) **Placebo**: Saline infusion and oral placebo; (2) **Ex-9**: Ex-9 infusion and oral placebo; (3) **sita**: Saline infusion and oral sitagliptin (sita); (4) **Ex-9/sita**: Ex-9 infusion and oral sita. Tablets with 100 mg sita or placebo in identical capsules were administered orally at 2200 h the evening before and at 0700 h on each study day. Ex-9 infusion protocol was the same as in study 1. The mixed-meal consisted of ½ slice of whole meal toast with 1 slice of cheese, margarine spread and marmalade, 2 dl yoghurt with glucose syrup, 20 g oatmeal, 16 raisins, and 5 almonds (total energy content: 1523 kJ, 53E% carbohydrate, 33E% fat, and 14E% protein) and was ingested evenly over 20 minutes. The mixed-meal with both semi-solid and solid components was chosen to replicate macronutrient composition of the liquid mixed-meal in study 1 and to mimic everyday food intake.

Blood was sampled frequently before and following the standard meal for a total of 4 h (**fasting**: -40, -35, -30; **basal**: -10, -5, 0; **postprandial**: 5, 10, 15, 20, 30, 45, 60, 90, 120, 180, and 240 min). After 240 min, an ad libitum meal was served as described above, and the patients were allowed to drink 50 mL of water after completion of the ad libitum meal. Infusion of Ex-9 or saline, blood sampling and appetite ratings were continued for 60 min (240-300 min).

Surgery

Standard RYGB was performed at the Department of Surgical Gastroenterology, Copenhagen University Hospital Hvidovre, Denmark, using a standard laparoscopic technique resulting in a pouch with a 75-cm-long biliopancreatic limb and a 125-cm-long Roux limb.

Peptides, drug and placebo

Ex-9 was prepared as previously described[20,21]. Sitagliptin (Januvia 100 mg, MSD, Denmark) or placebo was dispensed in identical capsules by the Capital Region of Denmark Pharmacy (Herlev, Denmark).

Visual Analogue Scale (VAS-) score

In both studies, VAS-scoring for hunger and satiety was performed at -30 min, 0 min and with 30-60 min intervals after the standard mixed meal. The VAS-scores were 100 mm with a text expressing the most positive and the most negative rating anchored at each end. Participants could not compare with previous ratings or discuss their ratings with others.

Sample collection and laboratory analyses

Plasma glucose was analyzed bed-side (YSI, Yellowstone). In *study 1*, EDTA plasma containing the DPP-4 inhibitor valine pyrrolidide (val-pyr) (0.01mmol/l, final concentration) was used for analysis of total (t) GLP-1 and glucagon as previously reported[20]. PYY_{3-36} was determined from EDTA plasma using a RIA kit (PYY 67-HK, Merck Millipore, Germany), CCK concentrations were measured in EDTA plasma[21], and insulin and C-peptide concentrations in serum as previously reported[20]. In *study 2*, EDTA plasma containing aprotinin (500 KIE/mL) and val-pyr was used for analysis of

tGLP-1, intact (i) GLP-1, glucagon, tPYY, PYY_{3-36}, CCK, oxyntomodulin and glicentin, and serum was used for analysis of C-peptide. DPP-4 activity was measured in EDTA-plasma without enzyme inhibitors as previously described[21]. Plasma concentrations of total amidated GLP-1, iGLP-1, glucagon, tPYY and PYY_{3-36} were determined by RIA after 70% extraction with ethanol as previously reported[23–26]. CCK was measured as previously reported[21]. Oxyntomodulin and glicentin were measured using a specific sandwich ELISA as describes elsewhere[10,27].

Calculations and statistical analyses

Data are presented as mean ± SE. Fasting hormone concentrations were calculated as the mean of fasting values (time -40 to -30 min), basal values as the mean of concentrations measured before the standard mixed meal (time -10 to 0 min). Total area-under the curve (tAUC) was calculated using the trapezoidal rule and incremental area-under-the-curve (iAUC) as the tAUC with subtracted basal values. In *study 1*, paired t-tests were used for testing differences before and after RYGB and for comparing responses between infusion of saline or Ex-9. Based on the results from *study 1* power calculations for *study 2* estimated a sample of 10 patients to show a 15% increased ad libitum food intake on the day with combined Ex-9 and DPP-4 inhibition (primary outcome) with a power of 80% and a two-sided significance level below 5%. In *study 2*, outcome measures were evaluated using linear mixed effects models with experimental day as fixed effect and individual patients as random effect. Logarithmic transformation was used if distribution was skewed (indicated in tables). In addition to the unadjusted model, the primary outcome (*ad libitum* food intake comparing placebo vs. Ex-9/sita) was further analyzed in two adjusted linear mixed effects models (both with individual subjects as random effect and experimental day as fixed effect): first

with adjustment for body-weight (fixed effect) and secondly with adjustment for study day number (fixed effect) since these co-variates affected ad libitum food intake in previous studies[18]. P-values <0.05 were considered significant. Calculations were performed using the R statistical software package (v 3.2.2).

Results

Study 1:

A total of 11 patients were included, two patients were excluded, thus nine patients (age 50±3 years; six men three women; BMI 39.2±2.5 kg/m^2, diabetes duration 5.7±1.3 years; supplementary table 1) completed the study as previously described[20]. In short, total BMI loss was 13.5±1.1% 3 months after RYGB, fasting glucose concentrations decreased and glucose tolerance improved (tAUC glucose: pre: 2.2±0.2 mol/L/min; 3 mo: 1.7±0.1)[20]. After RYGB antidiabetic treatment was discontinued in all patients. Fasting tGLP-1, glucagon and PYY concentrations did not change after RYGB, but fasting CCK decreased (Table 1). After RYGB basal glucagon concentrations decreased during saline infusion (p=0.02), but were unchanged during Ex-9[20]. Concentrations of tGLP-1, PYY_{3-36} and CCK were uninfluenced by the basal infusions.

Ad libitum food intake

Ad libitum food intake was 35% lower after compared to before RYGB surgery on the saline day (Pre: 2009±181 kJ; 3 mo: 1151±107 kJ, p=0.005). Before surgery, ad libitum food intake increased by ~16% on the day of Ex-9 infusion (+330±128 kJ) compared with saline (p=0.039), whereas food intake after surgery was uninfluenced by Ex-9 (p=0.71, Fig. 1).

Gut hormones and glucose

Secretion (tAUC) of tGLP-1, PYY_{3-36}, and CCK increased following the mixed meal after RYGB compared with before surgery (Table 1, Fig. 2). Preoperatively, Ex-9 did not affect PYY_{3-36} or CCK secretion, but tGLP-1 ($p=0.02$) and glucagon secretion increased slightly ($p<0.01$). After RYGB, effects of Ex-9 infusion on postprandial hormone secretion were more profound: tGLP-1 secretion increased by 59% ($p<0.01$), PYY_{3-36} secretion increased by 62% ($p<0.01$), glucagon secretion increased by 19% ($p<0.01$), and postprandial CCK secretion decreased by 31% ($p<0.01$) compared with the day of saline infusion.

VAS scores

Fasting state hunger and satiety did not change after RYGB (supplementary table 2). After RYGB, postprandial satiety was numerically increased (tAUC VAS satiety, $p=0.099$) and hunger reduced ($p=0.096$) during the 4 hour standard meal test comparing days with saline infusion. Neither before nor after surgery did Ex-9 affect hunger or satiety scores.

Study 2:

12 patients were included (age 36±7 years, four men eight women, BMI 33.5±6 kg/m^2, time from uncomplicated RYGB 5.4±1 months, all with normal glucose tolerance after RYGB but two with remission of preoperative type diabetes), as previously described[21]. In short, postoperative weight loss prior to first experimental day was 25±1 kg corresponding to a BMI loss of 19.6±1.2%. High and stable concentrations of Ex-9 were obtained and full DPP-4 inhibition was accomplished[21]. Neither fasting glucose nor fasting C-peptide concentrations differed between study days. Postprandial glucose

concentrations increased and insulin secretion decreased during Ex-9 but were unaffected by DPP-4 inhibition[21].

Ad libitum food intake

No effect on food intake was seen with either Ex-9 infusion or sitagliptin alone compared with placebo. However, combined Ex-9 infusion and sitagliptin increased ad libitum food intake by ~20% compared with placebo (placebo: 1304±115 kJ, Ex-9/sita: 1544±190 kJ, p=0.04 in the unadjusted model) (Fig. 1). The ad libitum meal was ingested in 16±1 minutes with no difference between experimental days. In the body-weight adjusted model the difference between Ex-9/sita vs. placebo remained significant with a p-value <0.01. Furthermore, ad libitum food intake increased on succeeding study days (p=0.01). In the model with adjustment for study day number, the difference in ad libitum food intake between Ex-9/sita vs placebo remained significant (p=0.04).

Fasting hormone concentrations

Fasting tGLP-1, tPYY, oxyntomodulin, CCK and glicentin did not differ between the 4 study days (Table 2). Sitagliptin administration increased concentrations of iGLP-1 compared with placebo, whereas PYY_{3-36} concentrations decreased. Basal concentrations of intact and total GLP-1 increased on days of Ex-9 infusion[21], while basal concentrations of tPYY, PYY_{3-36}, CCK, oxyntomodulin and glicentin were unaffected (data not shown).

Gut hormones in response to the standard mixed meal

Meal-induced tGLP-1 secretion (tAUC tGLP-1) increased on both days with Ex-9 infusion compared with placebo (both $p<0.01$) and decreased after sita administration ($p=0.02$) (Table 2, Fig. 3). Concentrations of iGLP-1 (tAUC iGLP-1) increased by 154%, 227% and 774% with Ex-9, sita and combined Ex-9/sita, respectively (all $p<0.01$ vs. placebo).

Ex-9 clearly increased tAUC PYY_{3-36} by 124% compared with placebo ($p<0.01$), whereas combined administration of Ex-9/sita decreased concentrations by 23% and 66% compared with placebo and Ex-9 alone, respectively ($p<0.01$ for both comparisons). Sita alone almost eliminated the PYY_{3-36} response. Total PYY increased on both days with Ex-9 infusions and decreased during sita alone.

Postprandial glucagon increased on both days with Ex-9 (both $p<0.01$ vs. placebo) and decreased with sita ($p=0.02$). Concentrations of oxyntomodulin also increased during Ex-9 ($p<0.01$), but *increased* significantly with sita, both compared with placebo ($p<0.01$) as well as Ex-9 infusions (Ex-9 vs. Ex-9/sita, $p<0.01$). Glicentin also rose on both days with Ex-9 infusion (both $p<0.01$ vs. placebo) but was unaffected by sita administration ($p=0.14$). Concentrations of CCK decreased 31% following Ex-9 ($p<0.01$) and were unaffected by sita.

Gut hormones in response to ad libitum meal

The patterns of responses of tGLP-1, iGLP-1, tPYY and PYY_{3-36} after the libitum meals were very similar to the patterns observed after the standard mixed meal in spite of the varying amounts of food ingested on the different study days (Table 3, Fig. 3). Only CCK numerically increased following the ad libitum meal during Ex-9 in contrast to a decrease after the standard mixed meal.

VAS scores

No differences were found when comparing VAS scores of hunger, satiety, nausea or pain between study days (supplementary table 3).

Discussion

In the present studies, we investigated the role of endogenous GLP-1 and PYY for the regulation of appetite and food intake in RYGB operated subjects. In s*tudy 1* patients with type 2 diabetes were studied with and without GLP-1R antagonism both before and after RYGB, where glucose tolerance was normalized. We found, that ad libitum food intake decreased substantially after surgery and that this was associated with an increased postprandial secretion of anorexigenic gut hormones including GLP-1 and PYY. This prospective finding confirms, what previous reports from cross sectional studies have indicated: that appetite measured by ad libitum food intake is reduced after RYGB[9]. However, contrary to our expectation, GLP-1R antagonism resulted in increased ad libitum energy intake only before but not after RYGB. Interestingly, examination of the postprandial gut hormone profiles during GLP-1R antagonism revealed a markedly altered secretion postoperatively, particularly for PYY secretion, which increased dramatically with the antagonist particularly *after* but not *before* RYGB. Since PYY is a very potent anorexigenic hormone, we speculated that this increased PYY secretion after RYGB might counteract the expected orexigenic effect of blocking the GLP-1R.

To test this hypothesis we designed *study 2* with the primary aim of investigating ad libitum food intake in RYGB-operated subjects during combined GLP-1R antagonism and DPP-4 inhibition to block formation of active PYY_{3-36}. In agreement with our

hypothesis, this combination triggered an increased food intake of ~20%, whereas no effect was seen with either GLP-1R blockage or DPP-4 inhibition alone. Again, GLP-1R antagonism alone markedly augmented concentrations of PYY_{3-36} (as in *study 1*), while active GLP-1 concentrations increased markedly during DPP-4 mediated lowering of PYY_{3-36} (compared with saline), probably counteracting effects on food intake on these experimental days. These changes in active PYY_{3-36} and GLP-1 make it impossible to identify the anorexigenic contributions of the individual hormones after RYGB. Nevertheless, it is possible to conclude that the two hormones do play an important role for the decreased food intake after the operation. These findings may also be interpreted to suggest that decreased energy intake after RYGB does not rely on the hypersecretion of just one gut hormone, but several (many), acting in concert to produce a powerful anorexigenic signal. However, it is not possible from these data to discern whether a combination is essential for significant inhibition (as suggested from co-infusion studies[17,18]) or whether the marked increases in the secretion of the unblocked hormone overshadowed the loss of effect of the blocked hormone.

GLP-1 and PYY are not the only hypersecreted anorexigenic hormones after RYGB, the list also includes oxyntomodulin[10,27], CCK[28], neurotensin and glucagon[29], while ghrelin secretion is suppressed[9,30]. In *study 2* concentrations of glucagon and oxyntomodulin increased during Ex-9/sita compared with placebo and this may possibly have counteracted further increases in food intake through activation of glucagon receptors. On the other hand, the effect size of ~20% increased food intake, during combined inhibition of GLP-1 and PYY_{3-36}, corresponds well with the effect seen during administration of exogenous GLP-1 and PYY to overweight, non-operated individuals[18]. That other hormones may participate in the regulation of food intake after

RYGB is supported by the approximately 50% increased food intake seen after octreotide administration; a similar effect was not seen in patients with gastric bands, another bariatric procedure that does not invoke changes in the postprandial gut hormone secretion profiles[11]. Similar results were recently obtained by *de Hollanda* et al., who examined the effects of octreotide on ad libitum food intake in RYGB patients with good and poor weight loss maintenance post-surgery and demonstrated increased ad libitum food intake by 50% in both groups[12]. However, the use of a somatostatin analogue, which inhibits numerous (not only gut) endocrine secretions and digestive functions makes it difficult to isolate the particular roles of gut hormones in any of these studies.

In the present studies, we were able to increase ad libitum food intake preoperatively (*study 1*) by Ex-9 infusion and post-RYGB during concomitant DPP-4 inhibition (*study 2*), demonstrating important effects of endogenously secreted GLP-1 in regulating food intake. Causality between endogenously secreted GLP-1 and reduced energy intake has not been established in human studies previously, and our findings are in variance with two recent reports that failed to show effects of GLP-1R antagonism on food intake in healthy lean subjects[31,32]. However, some noteworthy differences exist between those and our studies, since the infusion protocols differ. Melhorn et al. used an unprimed Ex-9 infusion with a rate of 750 pmol/kg/min and provided a fixed meal after 30 min; after further 90 min the ad libitum meal was served. Thus, Ex-9 was infused for a total of 120 min before the ad libitum meal was served, where maximum plasma concentrations might not have been reached as Ex-9 half-life is ~30 min[33]. The same argument could be made against the study by Steinert et al.[31], but here the ad libitum meal was served only 70 min after start of an unprimed infusion and the infusion rate was only 600

pmol/kg/min. As the dose-response relationship for the effect of GLP-1 on appetite regulation may differ from the effect on insulin secretion as seen with GLP-1R agonist therapy[34], the magnitude of plasma concentrations of Ex-9 obtained and the duration of blockade could be of critical importance for the effect on appetite. In the present studies, the bolus dose of Ex-9 instantly induced maximal Ex-9 concentration. Thus, more effective GLP-1R blockade for longer time could explain the success in increasing food intake in the present study. Further, our design with timing of the ad libitum meal following a fixed pre-load mixed-meal, which by itself affect hormone concentrations and potentially succeeding appetite, could contribute to the disparity in results.

PYY is secreted as full-length PYY_{1-36}, but is rapidly cleaved by DPP-4 to form the anorexigenic PYY_{3-36} which is selective for the satiety-promoting Y_2-receptor[35]. Thus, the anorexigenic action of PYY can be prevented by inhibiting the conversion of PYY_{1-36} to PYY_{3-36} by DPP-4 inhibition as in *study 2*[36]. However, DPP-4 inhibition may also affect the metabolism of other appetite regulating hormones. We found increasing concentrations not only of intact GLP-1 but also of oxyntomodulin on days with DPP-4-inhibition, whereas the precursor glicentin was unaffected, suggesting that oxyntomodulin may be a substrate for DPP-4. Provided that oxyntomodulin affects appetite in the concentrations reached achieved in *study 2* (which is not known), it could have influenced our results on food intake as mentioned above. The decreased concentrations of PYY_{3-36} during DPP-4 inhibition may explain the failure of DPP-4 inhibitors in general to promote weight loss in patients despite elevated levels of intact GLP-1.

The lack of Ex-9-effect after RYGB in *study 1* was indeed unexpected at first, and we have considered other possible explanations besides the concomitant hypersecretion of

PYY. Ex-9 was infused in adequate amounts to abolish insulin secretion after RYGB[21], but it may be a concern whether Ex-9 was able to reach GLP-1 receptors involved in appetite regulation in sufficient concentrations, due to possible differences in the dose-response relationships for glucose and appetite effects[34]. However, considering the effect of Ex-9 infusion on preoperative ad libitum intake and the effect of Ex-9 in combination with DPP-4 inhibition in *study 2*, we found insufficient GLP-1R blockade an unlikely explanation. Second, intact vagal signaling may be involved in GLP-1 induced appetite reduction; in humans, who have undergone truncal vagotomy with pyloroplasty, ad libitum food intake was uninfluenced by GLP-1 infusion[37]. Thus, damage to the vagal nerve during surgery could explain the lack of effect of Ex-9. However, vagal trunks are generally not injured during a routine RYGB, as also demonstrated by the preserved early response of postprandial pancreatic polypeptide, a marker of vagal signaling, which makes vagal nerve injury unlikely to explain the findings[29].

In conclusion, we found food intake to be decreased after RYGB along with increased secretion of the two appetite regulating L cell hormones, GLP-1 and PYY. Pharmacological blockade of actions from only one of these hormones had no effect on food intake after surgery, but resulted in concomitant increased secretion of the other, which may explain the missing effect on food intake. Combined blockade of GLP-1 and PYY actions increased food intake, supporting that exaggerated secretion of these hormones plays an important role for the decreased postoperative food intake. Our findings also support co-administration of GLP-1 and PYY as a therapeutic approach for treating obesity. Finally, our preoperative study indicates that endogenously secreted

GLP-1 plays an important role in regulating food intake in obese patients with type 2 diabetes.

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Conflict of interest:

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Figure legends

Figure 1

Food intake during an ad libitum meal test (served at t= 240) preceded by a 4-hour standard mixed meal test (at t=0) in two different studies: **(A)** 9 patients with type 2 diabetes examined before (Pre) and after (Post) RYGB on two days with and without infusion of the GLP-1R antagonist exendin9-39 (Ex-9) **(B)** 12 RYGB-operated patients examined on 4 days with placebo, Ex-9, the DPP-4 inhibitor sitagliptin (Sita) or Ex-9/Sita. Data are means ±SEM, * $p<0.05$ vs. placebo, #$p<0.05$ vs preoperatively.

Figure 2

Study 1. Plasma concentrations of gut-hormones in 9 patients with type 2 diabetes examined before (Pre) and 3 month after RYGB on two study days with mixed meal test including infusion of either saline or the GLP-1R antagonist Ex-9. Infusion of saline or Ex-9 was started at t=-30, a standard mixed meal was served at time=0. Data are means ±SEM. **(A)** Total GLP-1 **(B)** PYY_{3-36} **(C)** CCK. Grey solid lines (filled squares): Pre Saline; Grey dottet line (open squares) Pre Ex-9; Green solid lines (filled circles): 3 month Saline; Green dottet lines (open circles): 3 month Ex-9.

Figure 3

Study 2. Plasma concentrations of gut-hormones measured in 12 RYGB-operated patients on 4 different study days with either placebo, the GLP-1 receptor antagonist Exendin 9-39 (Ex-9), the DPP-4 inhibitor sitagliptin (Sita) or combined Ex-9/Sita. Infusion of saline or Ex-9 was started at t=-30, a standard mixed meal was served at time=0 and an ad libitum meal was served at time =240 (dotted line). **(A)** total PYY **(B)** PYY_{3-36} **(C)** total GLP-1 **(D)** intact GLP-1 **(E)** glucagon **(F)** CCK **(G)** oxyntomodulin **(H)** glicentin. Data are means ±SEM. Grey (squares): Placebo; Green (circles): Ex-9; Orange (triangles): Sita; Purple (diamonds):Ex-9/Sita.

Table 1. Gut-hormones in the fasting state and after the standard fixed mixed meal – *study 1*

	Pre		3 months	
	Saline	**Ex-9**	**Saline**	**Ex-9**
Fasting glucose (mmol l^{-1}) [a]	8.3±0.7	8.4±0.8	6.0±0.3**	6.2±0.3
Total GLP-1				
Fasting (pmol l^{-1}) [a]	9±1	8±1	7±1	7±1
tAUC (nmol l^{-1} × min)	2.4±0.3	3.0±0.3†	5.4±0.5**	8.6±1††
PYY $_{3-36}$				
Fasting (pmol l^{-1})	16±2	15±1	17±1	17±2
tAUC (nmol l^{-1} × min)	4.1±0.3	4.5±0.5	5.3±0.4**	8.6±1††
CCK				
Fasting (pmol l^{-1})	1±0	1±0	0.5±0**	0.6±0
tAUC (nmol l^{-1} × min)	0.46±0.1	0.54±0.1	0.70±0.1**	0.48±0.1††
Glucagon				
Fasting (pmol l^{-1}) [a]	23±3	24±3	21±2	22±2
tAUC (nmol l^{-1} × min)	5.7±1	6.5±1††	5.7±1	6.8±1††

Glucose and gut hormones in the fasting state and during a standard mixed meal test in nine patients with type 2 diabetes (study 1) before (Pre) and 3 months after RYGB (Roux-en-Y gastric bypass) with infusion of saline or the GLP-1 receptor antagonist Exendin 9-39 (Ex-9). * p<0.05, ** p<0.01 vs. corresponding preoperative value; † p<0.05, †† p<0.01 vs. saline (paired t-test)

[a] Data previously reported in ref[18]

Table 2. Gut-hormones in the fasting state and after the standard mixed meal – *study 2*

	Placebo	Ex-9	Sita	Ex-9/sita
Intact GLP-1				
Fasting (pmol l^{-1}) [b]	0.1 ± 0.1	0.4 ± 0.3	1.1 ± 0.4*	1.3 ± 0.6†
iAUC (pmol l^{-1} ×min) [a,b]	553 ± 100	1292 ± 279**	1477 ± 294**	3345 ± 597 **††
tAUC (pmol l^{-1}×min) [a]	598 ± 107	1391 ± 286**	1759 ± 332**	4353 ± 545**††
Total GLP-1				
Fasting (pmol l^{-1}) [b]	5.5 ± 0.7	5.2 ± 0.7	6.5 ± 0.6	6.5 ± 0.9
iAUC (pmol l^{-1} ×min) [b]	2838 ± 278	4918 ± 648**	1758 ± 394	4733 ± 538**
tAUC (pmol l^{-1} ×min) [a]	4198 ± 296	6518 ± 683**	2918 ± 384*	7303 ± 553**
Glucagon				
Fasting (pmol l^{-1}) [a,b]	21 ± 2	21 ± 2	20 ± 2	20 ± 3
iAUC (pmol l^{-1}×min) [b]	771± 194	1399 ± 253**	283 ± 167*	1195 ± 154
tAUC (pmol l^{-1}×min)	5379 ± 413	6492 ± 427**	4936 ± 426*	6155 ± 497**
PYY $_{3-36}$				
Fasting (pmol l^{-1})	2.2 ± 0.5	2.5 ± 0.4	1.1 ± 0.1*	1.1 ± 0.1*
iAUC (pmol l^{-1}×min)[a]	649 ± 171	1713 ± 137**	65.6 ± 56.7**	473 ± 189††
tAUC (pmol l^{-1}×min)[a]	1219 ± 143	2263 ± 208**	325 ± 58**	823 ± 203**††
Total PYY				
Fasting (pmol l^{-1})	9.4 ± 1.6	10.2 ± 2.1	8.8 ± 1.5	5.6 ±1.1
iAUC (pmol l^{-1}×min)	1037 ± 220	3447 ± 448**	745 ± 409	2968 ± 384**
tAUC (pmol l^{-1}×min)	3361 ± 363	5858 ± 617**	2054 ± 373*	4965 ± 608**
CCK				
Fasting (pmol l^{-1})[a]	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
iAUC (pmol l^{-1}×min)[a]	348 ± 72	209 ± 33**	439 ± 105	215 ± 49**
tAUC (pmol l^{-1}×min)[a]	439 ± 82	276 ± 37**	526 ± 121	294 ± 61 **
Oxyntomodulin				
Fasting (pmol l^{-1})	10.1 ± 1.2	10.2 ± 1.3	10.9 ± 1.5	11.4 ± 1.5
iAUC (pmol l^{-1}×min)	4311 ± 892	7770 ± 1031**	9327 ± 1172**	11514 ± 1193**††
tAUC (pmol l^{-1}×min)	7512 ± 829	11010 ± 947**	12607 ± 1234**	14874 ± 1097**††
Glicentin				
Fasting (pmol l^{-1}) [a]	21 ± 2	23 ± 4	27 ± 3	24 ± 3
iAUC (pmol l^{-1}×min)	23527 ± 3182	34054 ± 3742**	19486 ± 3108	34879 ± 3461**
tAUC (pmol l^{-1}×min)	28 947 ± 3448	41 174 ± 3391**	25 706 ± 3510	44 559 ± 3323**

Gut-hormone concentrations in the fasting state and after a mixed-meal test (t=0-240) in 12 subjects after RYGB investigated on 4 different study days with either placebo, the GLP-1 receptor antagonist Exendin 9-39 (Ex-9), the DPP-4 inhibitor sitagliptin (Sita) or combined Ex-9/Sita. Data are means ±SEM. *p<0.05 and **p<0.01 compared with placebo. †p<0.05 and ††p<0.01 Ex-9 vs. Ex-9/Sita. [a] Logarithmic transformation [b] Data previously reported in ref [19]

Table 3. Gut-hormone concentrations after the ad libitum meal – *study 2*

	Placebo	Ex-9	Sita	Ex-9/Sita
Intact GLP-1				
$tAUC_{240\text{-}300}$ (pmol l^{-1} × min) [a]	44.6 ± 15	135 ± 38 *	184 ± 40 **	431 ± 60 **††
Total GLP-1				
$tAUC_{240\text{-}300}$ (pmol l^{-1} × min) [a]	369 ± 41	580 ± 95**	317 ± 37	674 ± 89**
Glucagon				
$tAUC_{240\text{-}300}$ (pmol l^{-1} × min) [a]	664 ± 55	850 ± 67**	642 ± 64	779 ± 71**†
$PYY_{3\text{-}36}$				
$tAUC_{240\text{-}300}$ (pmol l^{-1} × min) [a]	114 ± 19	158 ± 37	37.5 ± 3.9**	56.3 ± 13**††
Total PYY				
$tAUC_{240\text{-}300}$ (pmol l^{-1} × min) [a]	400 ± 57	574 ± 121	275 ± 43*	458 ± 94
CCK				
$tAUC_{240\text{-}300}$ (pmol l^{-1} × min) [a]	31.8 ± 5.6	50.3 ± 15	51.1 ± 16	14.8 ± 14*

Gut-hormone concentrations following the ad libitum meal (time=240-300 min) on the 4 different study days with either placebo, the GLP-1 receptor antagonist Exendin 9-39 (Ex-9), the DPP-4 inhibitor sitagliptin (Sita) or combined Ex-9/Sita. Duration of the ad libitum meal was 16 ±1 minutes. Data are means ±SEM. *p<0.05 and **p<0.01 vs. placebo. †p<0.05 and ††p<0.01 Ex-9 vs. Ex-9/sita. [a] Logarithmic transformation